prepared from a number of clones and several mutants lacking BamHl sites identified by restriction digestion.- -

Please replace the paragraph on page 120, lines 15-22, with the following rewritten paragraph:

- -The oligonucleotide G3 Bamlink was designed to introduce a BamHl site at a number of possible sites within the peptide linker sites A and B, see figure 16(ii). The sequence of the linker is: Bamlink 5'CC (G or A) CC ACC CTC GGA TCC (G or A) CC ACC CTC 3' (SEQ ID NO:14). Its relationship to the peptide repeats in gene III is shown in figure 16.- -

Replace the paragraph on page 121, lines 1-16 with the following paragraph:

- - 1. cDNA is prepared from spleen RNA from an appropriate mouse and the VH and VLK repertories individually amplified. Separately, primers reverse and complementary to VH1FOR-2 (domain 1) and VLK2BACK (domain 2) are used to amplify an existing scFv-containing DNA by PCR. (The term FOR refers to e.g. a primer for amplification of sequences on the sense strand resulting in antisense coding sequences. The term BACK refers to e.g. a primer for amplification of sequences on the antisense strand resulting in sense coding sequences). This generates a 'linker' molecule encoding the linker with the amino acid sequence (1 letter code) $(GGGGS)_3$ (SEQ ID NO:15) which overlaps the two primary (VH and VLK) PCR products. - -

Please replace the paragraph on page 126, lines 14-20, with the following rewritten paragraph:

- - The primers anneal to the 3' end. Examples of kappa light chain primers are MJK1FONX, MJK2FONX, MJK4FONX and MJK5FONX (provided under 'Primer Sequences' below) and examples of heavy chain primers are MIGG1, 2 (CTG GAC AGG GAT CCA GAG TTC CA) (SEQ ID NO:16) and MIGG3 (CTG GAC AGG GCT CCA TAG TTC CA) (SEQ ID NO:17) which anneal to CH1.- -

by the variety of digestion patterns seen in Figure 23(i) and Figure 23(ii), and sequencing revealed the presence of most VH groups (R. Dildrop, Immunol. Today 5 85-86. 1984) and VK subgroups (Kabat. E.A. et al. 1987 supra) (data not shown). None of the 568 clones tested bound to phOx as detected by ELISA as in example 9.- -

Please replace the paragraph bridging page 147 (starting at line 27) through page 148 (line 11) with the following rewritten paragraph:

- - To sequence clones, template DNA was prepared from the supernatants of 10 ml cultures grown for 24 hours, and sequenced using the dideoxy method and a Sequenase kit (USB), with primer LINKFOR (see example 14) for the VH genes and primer fdSEQ1 (5'-GAA TTT TCT GTA TGA GG -3') (SEQ ID NO:36) for the Vk genes. Twenty-three of these hapten-binding clones were sequenced and eight different VH genes (A to H) were found in a variety of pairings with seven different Vk genes (a to g) (Fig. 24). Most of the domains, such as VH-B and Vk-d were 'promiscuous', able to bind hapten with any of several partners.- -

VW 1/11/10

Please replace the paragraph bridging page 148 (starting at line 10) through page 149 (line ~~9~~) with the following rewritten paragraph:

- -The sequences of the V-genes were related to those seen in the secondary response to phOx, but with differences (Fig. 24). Thus phOx hybridomas from the secondary response employ somatically mutated derivatives of three types of Vk genes - Vkoxl. 'Vkox-like' and Vk45.1 genes (C. Berek, G. M. Griffiths & C. Milstein Nature 316 412-418 (1985). These can pair with VH genes from several groups, from Vkoxl more commonly pairs with the VHoxl gene (VH group 2. R.Dildrop uupra). Vkoxl genes are always, and Vkox-like genes often, found in association with heavy chains (including VHoxl) and contain a short five residue CDR3, with the sequence motif Asp-X-Gly-X-X (SEQ ID NO:37) in which the central glycine is needed to create a cavity for phOx. In the random combinatorial library however, nearly all of the VH genes belonged to group 1, and most of the Vk genes were ox-like and associated with VH domains with a five residue CDR3, motif Asp/Asn-X-Gly-X-X (SEQ ID NO:38) (Fig. 24). Vkoxl and VHoxl were found only once (Vk-f and VH-E), and not in combination with each other. Indeed Vk-f lacks the Trp91 involved in phOx binding and was paired with a VH (VH-C) with a six residue CDR3.- -

VH1BACKSFI15, 5'-CAT GCC ATG ACT CGC GGC CCA GCC GGC CAT GGC C(C/G)A GGT (C/G)(A/C)A (A/G)CT GCA G(C/G)A GTC (A/T)GG-3' (SEQ ID NO:45);

FABNOTFOH, 5'-CCA CGA TTC TGC GGC CGC TGA AGA TTT GGG CTC AAC TTT CTT GTC GAC-3' (SEQ ID NO:46);

FABNOTFOK, 5'-CCA CGA TTC TGC GGC CGC TGA CTC TCC GCG GTT GAA GCT CTT TGT GAC-3' (SEQ ID NO:47);

MVKBAAPA, 5'-CAC AGT GCA CTC GAC ATT GAG CTC ACC CAG TCT CCA-3' (SEQ ID NO:48);

MVKBASFI, 5'-CAT GAC CAC GCG GCC CAG CCG GCC ATG GCC GAC ATT GAG CTC ACC CAG TCT CCA-3' (SEQ ID NO:49);

VK3F2NOT, 5'-TTC TGC GGC CGC CCG TTT CAG CTC GAG CTT GGT CCC -3' (SEQ ID NO:50).

Restriction sites are underlined.

Rescue of Phage and Phagemid particles - -

VW 1/11/10

Please replace the paragraph bridging page 160 (starting at line 24) through page 161 (line 7) with the following rewritten paragraph:

- - The phagemid vector, pHEN1 (fig. 26(a)), is based upon pUC119 and contains restriction sites (SfiI and NotI) for cloning the fusion proteins. Here the transcription of antibody-g3p fusions is driven from the inducible lacZ promoter and the fusion protein targetted to the periplasm by means of the pelB leader. Phagemid was rescued with VCSM13 helper phage in 2xTY medium containing no glucose or IPTG: under these conditions there is sufficient expression of antibody-g3p. Fab and scFv fragments of NQ10.12.5 cloned into pHEN1 for display were shown to bind to phOx-BSA (but not BSA) by ELISA (Table 5) using the same criterion as above.- -

VW 1/11/10

On page 179, please replace lines 10-16 with the following rewritten paragraph:

- -The construct fdphoAla166 (derived in example 11) was converted back to the wild type residue (arginine) at position 166 by in vitro mutagenesis (Amersham International) using the printer APARG166:5' TAGCATTTGCGCGAGGTCACA 3' (SEQ ID NO:51).

This construct with the wild type insert was called fdphoArg166.

On page 183, please replace lines 1-17, with the following rewritten paragraph:

catalytic activity is measured in preparations of vector phage from either TG1 or KS272 cells (Table 7), indication that the catalytic activities above are due to phage enzyme and not contamination with bacterial phosphatase. Addition of phage particles to soluble enzyme does not have a significant effect on activity (Table 7). - -

On page 189, please replace lines 9-15, with the following

- - VK-TERM-FOR

5' TGG AGA CTG GGT GAG CTC AAT GTC GGA GTG AGA ATA GAA AGG 3' (SEQ ID NO:52) (overlapping with VK2BACK [example 14])

and

CH1-TERM-BACK

5'AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA GTT GAG CCC AAA TCT AGC TGA TAA ACC GAT ACA ATT AAA GGC 3' (SEQ ID NO:53) (overlapping with HuIgG1-4 CH1-FOR)- -

VW 1/11/10

4 Please replace the paragraph bridging page 191 (starting at line 28) through page 192 (line ~~5~~) with the following rewritten paragraph:

- - Aliquots of the ligation reaction were transformed into competent TG1/pUC19gIII and plated on SOB medium containing ampicillin at 100μg/ml and kanamycin at 50μg/ml. Colonies were screened for the presence of a deletion by PCR with primers gIIIFUBA and KSJ12 (CGGAATACCCAAAAGAACTGG)(SEQ ID NO:54).- -

VW 1/11/10

On page 192, please replace lines 5-~~16~~ 17, with the following rewritten paragraph:

- - KSJ 12 anneals to gene VI which is immediately downstream of gIII in the phage genome, so distinguishing gIII on the helper phage from that resident on the plasmid. Three clones gave trunctated PCR products corresponding to deletions of ca. 200, 400 and 800bp. These clones were called M13K07 gIII Δ Nos 1,2 and 3 respectively. No clones were isolated from the earlier Bal 31 time points, suggesting that these are in some way lethal to the host cell. Several clones were isolated from later time points, but none of these gave a PCR product, indicating that the deletion reaction had gone too far.- -